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Interplay between MEK and PI3 kinase signaling regulates the subcellular localization of protein kinases ERK1/2 and Akt upon oxidative stress

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ABSTRACT

ERK and Akt kinases are key components that participate in numerous regulatory processes, including the response to stress. Using novel tools for quantitative immunofluorescence, we show that oxidant exposure controls the intracellular activation and localization of ERK1/2 and Akt. Oxidative stress alters the nuclear/cytoplasmic levels of the kinases, drastically changing phospho-ERK1/2 and phospho-Akt(Ser473) levels in the nucleus. Moreover, pharmacological inhibition of PI3 kinase modulates the intracellular distribution of phospho-ERK1/2, whereas MEK inhibition affects phospho-Akt(Thr308) and phospho-Akt(Ser473). Our studies identify a new signaling link in the nucleus of stressed cells, where changes in phospho-ERK1/2 levels correlate directly with changes in phospho-Akt(Ser473).

1. Introduction

Signaling through PI3 → Akt and MEK → ERK1/2 modules is essential for cell growth, proliferation and survival of different forms of stress. Various stimuli trigger the activation of Akt and ERK1/2, thereby inducing the phosphorylation of distinct target molecules located in the cytoplasm and in the nucleus. The specificity of signaling events not only depends on the activation of kinases, but also on their proper location. This is indicated by the fact that many kinases, phosphatases and their targets relocate within the cell upon changes in physiology [1–6]. Activation of the MAP kinases ERK1/2 by dual phosphorylation (here referred to as p-ERK1/2) is linked to the nuclear accumulation of the kinases [1]. Full activation of Akt requires the phosphorylation of both Thr308 and Ser473 (here referred to as p-Akt308 and p-Akt473) [7–9]. Thr308 is modified by PDK1; mTORC2 is the most prominent kinase that phosphorylates Ser473, but other kinases have been implicated in Ser473 modification as well [8–15]. Several publications suggest that the signaling events and cell type can determine which kinase phosphorylates Ser473, and some of the pathways leading to Ser473 phosphorylation are insensitive to PI3 kinase inhibitors [10–13,15]. Interestingly, recent data demonstrate that particular pools of activated Akt can be generated in the cytoplasm with regulators that are restricted to this compartment, whereas unique functions can be attributed to nuclear Akt [16,17].

Upon exposure to certain stimuli and for several types of cancer, signaling through both PI3 kinase and MEK → ERK modules is activated simultaneously (reviewed in [18–21]). As the activation of these two signaling cascades is likely to have different downstream effects, it is important to regulate the relative input from either pathway to achieve the appropriate response. This may occur by cross-talk between both signaling routes [22], adding more complexity to the spatio-temporal control of signaling. Of particular importance to the balancing of different signaling cascades and the ultimate downstream effects is the control of kinase levels in different cellular compartments, especially in the nucleus and cytoplasm.

Oxidative stress is crucial to human health as it is linked to a large number of diseases and pathologies, including diabetes, obesity and ischemia/reperfusion injury of the heart and brain [23–25]. In the studies presented here, we used diethyl maleate as an oxidant to analyze in a quantitative fashion the effects on ERK1/2 and Akt activation and localization. Furthermore, with pharmacological inhibitors of PI3 and MEK-mediated signaling we tested the hypothesis that interplay between these pathways affects kinase activation and localization. For the experiments described here, we took advantage of recent developments in imaging and image-analysis to monitor in a quantitative fashion changes in ERK1/2 and Akt kinase compartmentalization. The significance of Akt and MEK signaling were monitored by measuring the

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nucleocytoplasmic distribution of the transcription factor FoxO3a, a downstream target of both signaling pathways.

2. Materials and methods

2.1. Growth of cells, stress treatment and incubation with pharmacological inhibitors

HeLa cells were grown on poly-lysine coated cover slips and treated with diethyl maleate as described [3]. For treatment with pharmacological inhibitors, cells were pre-incubated for 1 h with the solvent DMSO (D), 50 μM LY294002 (LY), 25 μM PD98059 (PD) or a combination of both drugs (Calbiochem) followed by treatment with ethanol (controls) or 2 mM diethyl maleate (for 4 h at 37 °C). DMSO or inhibitors were present throughout the incubation with the solvent ethanol or DEM. Results were obtained for at least three independent experiments.

2.2. Antibodies

The following antibodies were used for immunofluorescence: dually phosphorylated ERK1/2 (p-ERK1/2; Cell Signaling, #9106), total ERK1/2 (t-ERK1/2; StressGen, KAP-MA001C), phosphorylated Akt-Thr308 (p-Akt308; Cell Signaling, #2965), phosphorylated Akt-Ser473 (p-Akt473; Santa Cruz Biotechnology, sc-7985), total Akt (t-Akt; Santa Cruz Biotechnology, sc-1619; Cell Signaling, #9272), FoxO3a (Cell Signaling; #2497); HuR (Santa Cruz Biotechnology, sc-5261); rp S6 (Santa Cruz Biotechnology, sc-13007); G3BP (BD Transduction Laboratories, # 611126). Antibodies were diluted as recommended by the suppliers. Antibodies against p-Akt308 and p-Akt473 are likely to recognize Akt independent of the phosphorylation state at residue Ser473 or Thr308, respectively. FITC- or Cy3-labeled secondary antibodies were generated in donkeys and affinity-purified to minimize cross-reactivity (Jackson ImmunoResearch).

2.3. Immunofluorescent staining and confocal microscopy

Established protocols were used for the fixation and permeabilization of cells and incubation with primary and secondary antibodies [5]. Confocal images were collected with a Zeiss LSM510 in the multi-track mode for an optical slice thickness of >0.7 μm. p-ERK1/2 and t-ERK1/2 were detected with FITC- and Cy3-labeled secondary antibodies, respectively. Settings for pinhole and detector gain were identical within each experiment and proper filters were used to minimize cross-talk between the channels.

2.4. Quantification of fluorescent signals

Quantification of pixel intensities in nuclear and cytoplasmic compartments was as described [5]. In brief, nuclear and cytoplasmic fluorescence was measured using the multiwavelength cell scoring module following recently developed protocols that have been described in detail [5]. For each condition the distribution of total and activated kinases was quantified for at least 50 cells. Bar graphs in Figs. 1–3 depict pixel intensities/area. Pixel intensities/area obtained for control conditions (DMSO, EtOH) were defined as 1. All changes in the pixel intensities/area are expressed relative to the control condition. For example, an increase to 1.6-fold indicates that the pixel intensity/area is 1.6 times the value observed in control cells.

2.5. Detection of polyA$^+$ RNA

PolyA$^+$ RNA was localized by hybridization with Cy3-labeled oligo dT(50) (Gene Link). Control and stressed cells were washed with PBS and fixed with 3.7% formaldehyde in PBS (15 min, room temperature), permeabilized in 0.3% Triton-X 100/PBS (10 min, room temperature) and washed in PBS. Following 15 min incuba-

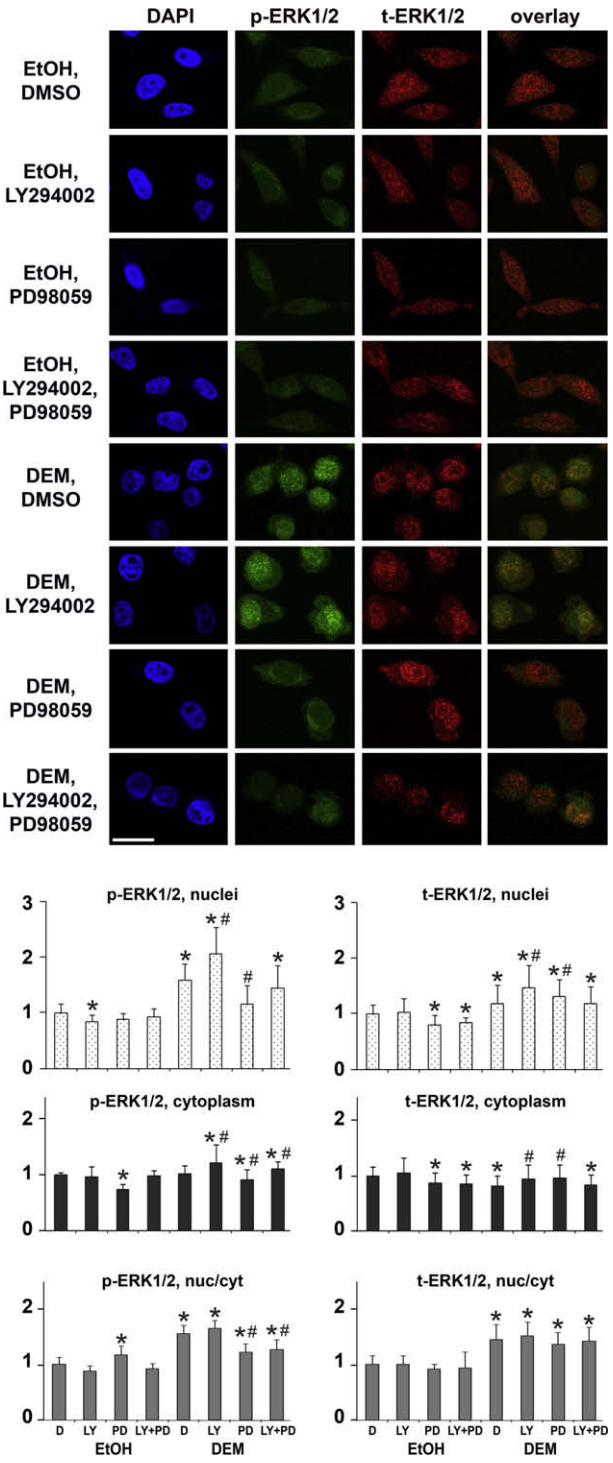

Fig. 1. Oxidative stress, PI3 kinase and MEK → ERK signaling alter the localization of activated ERK1/2. The localization of p-ERK1/2 and t-ERK1/2 was monitored by indirect immunofluorescence followed by confocal microscopy under the different conditions shown in the figure. Pixel intensities in nuclear and cytoplasmic compartments were quantified for dually phosphorylated and total ERK1/2. Nuclear and cytoplasmic fluorescence was quantified using the multiwavelength cell scoring module; for each condition the distribution of total and activated kinases was measured for at least 50 cells. The graphs depict pixel intensities/area. One-way ANOVA was used for multiple comparisons between all groups, with EtOH/DMSO treated cells as reference; * indicates $P < 0.05$. For comparisons of stressed cells, DEM/DMSO treatment served as reference and # denotes $P < 0.05$. Size bar is 20 μm.

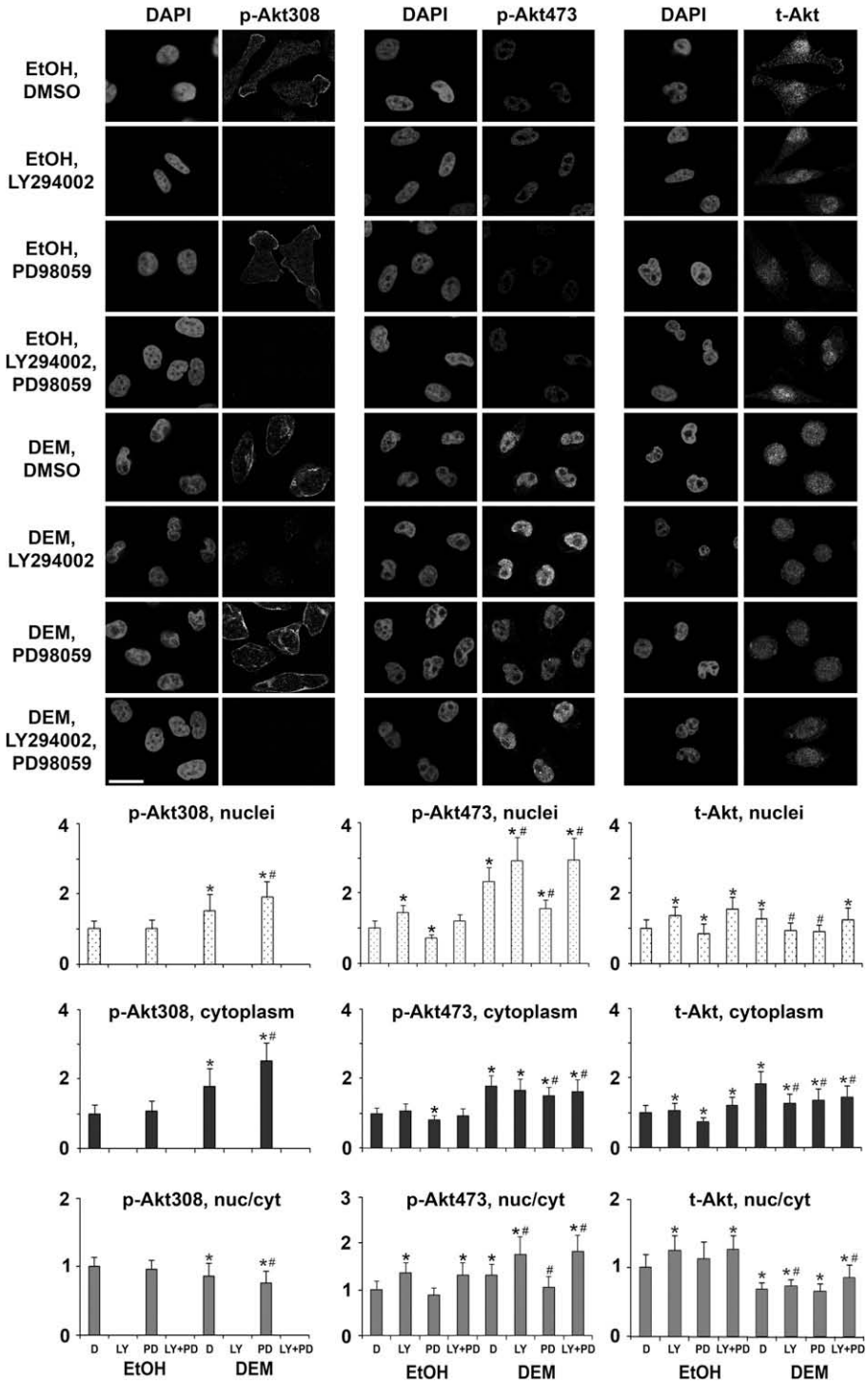

Fig. 2. The localization of Akt is regulated by oxidative stress as well as signaling through PI3 kinase and MEK → ERK. The levels of nuclear and cytoplasmic p-Akt308, p-Akt473 and t-Akt were quantified under different non-stress and stress conditions. The acquisition of confocal images, quantification of fluorescence intensities in the nuclear and cytoplasmic compartments was carried out as described for Fig. 1. Size bar is 20 μm.

tion in pre-hybridization buffer at 37 °C (2× SSC, 20% formamide, 2 mg/ml BSA, 1 mg/ml yeast tRNA), samples were hybridized overnight at 37 °C in pre-hybridization buffer containing 10% dextran sulfate and 1 nmol/ml Cy3-oligo-dT(50). Samples were washed in 2× SSC, 20% formamide (5 min, 42 °C), 2× SSC (5 min, 42 °C), 1× SSC (5 min, room temperature), PBS (5 min, room temperature), stained with DAPI and mounted in Vectashield.

2.6. Statistical analyses

Data were analyzed by one-way ANOVA to determine differences among groups. Multiple comparisons were carried out with control cells (EtOH, DMSO) as reference; * in Figs. 1–3 denotes $P < 0.05$. For multiple comparisons among stressed samples, cells incubated with DEM and DMSO served as reference; for these comparisons # indicates $P < 0.05$.

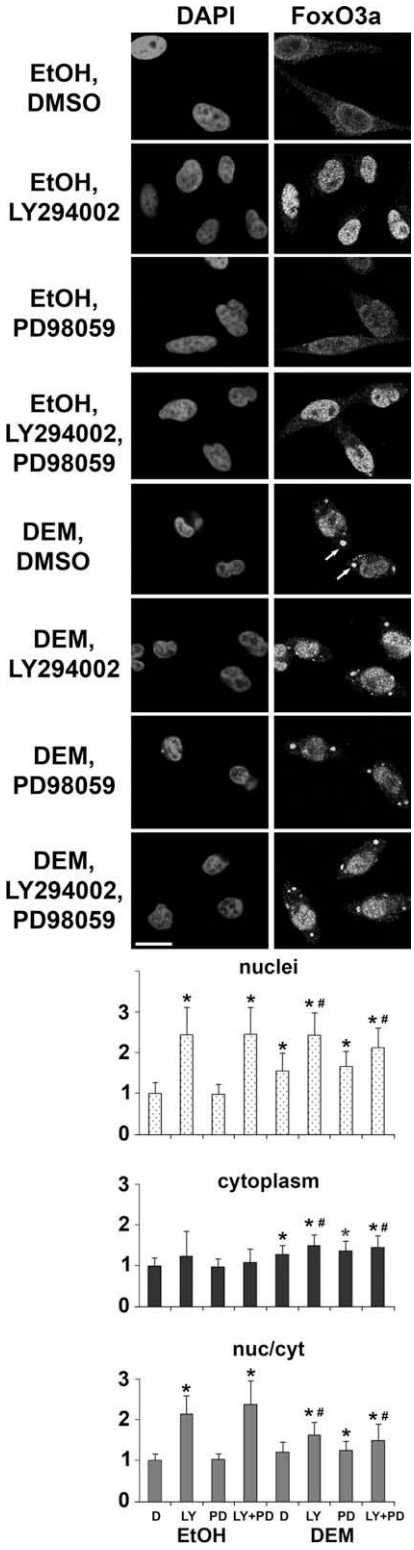

Fig. 3. The intracellular localization of transcription factor FoxO3a is controlled by stress and signaling through PI3 kinase and MEK → ERK1/2 pathways. HeLa cells were treated as in Fig. 1 and images were acquired by high-throughput screening technology [3,5]. Nuclear and cytoplasmic fluorescence was quantified using the MetaXpress multi wavelength cell scoring module; for each condition FoxO3a distribution was measured for at least 50 cells. Examples of SGs are marked with arrows; size bar is 20 μm. Note that in stressed cells nuclear FoxO3a levels are significantly different for treatment with LY294002 (DEM/LY) as compared to the incubation with both LY294002 and PD98059 (DEM/LY + PD).

3. Results

3.1. Oxidative stress alters the intracellular distribution of ERK1/2 and phosphorylated Akt

Cell fractionation is frequently used to determine the association of proteins with different organelles, a method that is tedious and prone to errors, in particular for proteins that bind to large cellular structures such as the filament systems. Moreover, procedures required to obtain pure fractions may lead to artificial phosphorylation or dephosphorylation of the protein of interest. Importantly, cell fractionation can not provide information on whether a component present in nuclear fractions is inside the nucleus, bound to the nuclear envelope or associated with the cytoplasmic side of the nuclear membranes. However, this knowledge is important to determine whether a kinase can access its substrates inside the nucleus. This is of particular interest to the analysis of ERK1/2 and Akt, since these kinases have numerous targets, in both the nuclear and cytoplasmic compartments.

To examine the spatial distribution of ERK1/2 and Akt kinases and detect subtle changes in their localization, we used recently developed protocols for the quantitative analysis of protein localization [5]. These procedures combine indirect immunofluorescence, confocal imaging and computer-based image-analysis to measure the *intra*nuclear and cytoplasmic levels of a particular protein [3–5].

With the immunofluorescence methods described above, the amounts of p-ERK1/2, t-ERK1/2, p-Akt308, p-Akt473 and t-Akt were quantified inside the nucleus and in the cytoplasm. Figs. 1 and 2 show the results for these experiments; the quantification is depicted as pixel intensities/area. Results were normalized to non-stress control conditions, which were defined as 1. All the numbers below depict the changes relative to the unstressed control. [For example, a 1.6-fold change in the nucleus denotes that the concentration in the nucleus was 1.6 times the amount detected in control nuclei; 1.0-fold means that there was no change relative to the control, and 0.8-fold indicates that the level decreased to 0.8 of the amount in control cells.]

In response to DEM treatment without kinase inhibitors (Figs. 1 and 2), the levels in nuclei increased significantly for p-ERK1/2 (to 1.6-fold, compare samples labeled D/EtOH with D/DEM), p-Akt308 (to 1.5-fold) and p-Akt473 (to 2.3-fold). In the cytoplasm, oxidative stress elevated the concentration of p-Akt308 (to 1.8-fold), p-Akt473 (to 1.8-fold) and t-Akt (to 1.8-fold), but not of p-ERK1/2 (1.0-fold) or t-ERK1/2 (to 0.8-fold).

We next tested whether the rise in nuclear p-ERK1/2 and p-Akt can be attributed to changes in the t-ERK1/2 and t-Akt distribution. Indeed, stress increased the levels of t-Erk1/2 (to 1.2-fold) and t-Akt (to 1.3-fold) in nuclei (Figs. 1 and 2). (Note that due to the different volumes of the nuclear and cytoplasmic compartments an increase in nuclear t-Akt will not lead to the same decrease in cytoplasmic levels of the kinase.) These results are consistent with the interpretation that a portion of ERK1/2 and Akt that was activated in the cytoplasm moves to the nucleus following oxidant exposure. Alternatively, ERK1/2 and Akt may first relocate to the nucleus to become subsequently phosphorylated.

Since the relative abundance of activated kinases in the nucleus and cytoplasm is likely to impact the endpoint of signaling, we determined whether their nuclear/cytoplasmic ratio changed in response to oxidant exposure (Figs. 1 and 2; bottom panels, nuc/cyt). This analysis revealed a significant rise in the nuclear/cytoplasmic ratio for p-ERK1/2 (to 1.6-fold), t-ERK1/2 (to 1.45-fold) and p-Akt473 (to 1.3-fold), whereas a reduction was observed for p-Akt308 (to 0.9-fold) and t-Akt (to 0.7-fold).

3.2. Inhibition of PI3 kinase and MEK activation regulates the distribution of kinases in stressed cells

Given that both PI3 kinase and MEK → ERK signaling cascades were activated by the oxidant DEM, we next tested whether pharmacological inhibition of PI3 kinase and MEK activation affected the intracellular distribution of ERK1/2 and Akt. The inhibitors LY294002 and PD98059, which target PI3 kinase or MEK1/2 and MEK5, respectively, were used as pharmacological tools to measure potential changes in the levels of kinases in the nuclear and cytoplasmic compartments.

Under non-stress conditions, p-Akt308 associates with the plasma membrane where it interacts with cortical F-actin [26, and references therein]. Likewise, in our studies p-Akt308 at the cell periphery partially co-localized with filamentous actin, as evident by staining with fluorescently labeled phalloidin (Supplementary Fig. 1). This partial co-localization was also observed when MEK signaling was inhibited with PD98059.

In stressed cells, LY294002 elevated the levels of p-ERK1/2 (to 2.1-fold), t-ERK1/2 (to 1.5-fold) and p-Akt473 (to 2.9-fold) in nuclei of stressed cells, but had a lesser effect on the cytoplasmic kinases (Figs. 1 and 2, LY/DEM).

A different scenario was observed for the MEK inhibitor PD98059, which increased p-Akt308 in nuclei (from 1.5-fold upon DEM treatment to 1.9-fold in DEM/ PD98059 incubated cells) and cytoplasm (from 1.8-fold to 2.6-fold). Interestingly, PD98059 had the opposite effect on p-Akt473, which decreased in the nuclear compartment of stressed cells (from 2.3-fold to 1.6-fold), whereas t-ERK1/2 changed from 1.2-fold to 1.3-fold and t-Akt from 1.3-fold to 0.9-fold. As expected, PD98059 reduced the levels of p-ERK1/2 in nuclei of stressed cells. When LY294002 was combined with PD98059, the effect of MEK inhibition on nuclear p-ERK1/2 and p-Akt473 was at least partially overcome by preventing PI3 kinase activation.

Taken together, our data suggest that PI3 kinase and MEK-dependent signaling control the distribution of p-ERK1/2, p-Akt308 and p-Akt473 under stress conditions (Table 1). Results obtained for the combination of PI3 kinase and MEK inhibitors further substantiate our hypothesis and support the model that these processes are controlled by the interplay between different signaling cascades. In particular, a direct correlation was observed for changes of p-ERK1/2 and p-Akt473 in the nucleus of stressed cells.

3.3. Effect of Akt and MEK-dependent signaling on the distribution of FoxO3a

To determine the consequences of stress as well as MEK and PI3 kinase signaling we have chosen FoxO3a, which is an authentic target of both signaling pathways. Members of the forkhead family of transcription factors (FoxO), such as FoxO3a, are key players in the regulation of gene expression under various physiological conditions [27,28]. Crucial to the control of FoxO-dependent transcription is the proper subcellular localization of FoxO proteins, which we analyzed here in a quantitative and systematic fashion. Fig. 3 shows FoxO3a distribution in control and stressed cells as well as the outcome of pharmacological kinase inhibition. In unstressed cells, the PI3 kinase inhibitor LY294002 increased the amount of FoxO3a in nuclei to 2.4-fold, whereas the MEK inhibitor PD98059 had no effect. Oxidative stress raised FoxO3a levels in nuclei to 1.5-fold; this was further augmented by PI3 kinase inhibition which increased nuclear levels to 2.4-fold. Collectively, our data suggest that activated Akt, known to promote FoxO3a nuclear export, is the limiting factor for nuclear accumulation of FoxO3a under stress conditions.

Data described above demonstrated cross-talk between MEK and PI3 kinase signaling which regulated the activation and localization of Akt and ERK1/2. Importantly, this interplay also affected the nuclear accumulation of FoxO3a, but was limited to oxidant-treated cells. The profile of DEM-induced changes in nuclear FoxO3a levels with or without kinase inhibitors was similar to those observed for p-Akt473 and p-ERK1/2. Moreover, simultaneous inhibition of PI3 and MEK kinases led to a statistically significant reduction of FoxO3a levels in nuclei when compared to PI3 kinase inhibition only ($P < 0.05$; multiple comparisons among DEM-treated cells with DEM/LY294002 as reference). Taken together, this suggests that PI3 kinase is the most important factor determining FoxO3a localization under normal and stress conditions, whereas signaling through MEK is not a limiting factor under non-stress conditions. However, in stressed cells and in the absence of PI3 kinase activation, MEK signaling contributes to FoxO3a distribution as well.

Surprisingly, DEM treatment not only changed the nuclear concentration of FoxO3a, but also altered its distribution in the cytoplasm, where FoxO3a became associated with granules (Fig. 3; arrows). Many forms of stress cause the formation of cytoplasmic stress granules (SGs), compartments that contain mRNA, RNA-binding and small ribosomal subunit proteins [29]. Indeed, following DEM incubation FoxO3a co-localized with HuR, G3BP1 and ribosomal protein S6 in cytoplasmic SGs, which were also enriched for polyA$^+$ RNA (Supplementary Fig. 2), suggesting a possible role for FoxO3a in the cytoplasm. In this scenario, the proper balance between nuclear and cytoplasmic levels would be important for FoxO3a functioning in both compartments, and we calculated the nuclear/cytoplasmic ratios for all experimental conditions (Fig. 3; bottom panels; nuc/cyt). The ratio was somewhat increased by stress (to 1.2-fold), but the most pronounced change was seen in unstressed cells following the inhibition of PI3 kinase (to 2.1-fold) or of both PI3 kinase and MEK (to 2.3-fold).

Collectively, our results demonstrate that PI3 kinase signaling is the most important component that determines FoxO3a localization. Nevertheless, upon oxidative stress, but not in unstressed cells, the nucleocytoplasmic FoxO3a distribution is also regulated by an interdependent network of PI3 kinase and MEK signaling. Moreover, our data show, for the first time, that oxidant treatment triggers the association of FoxO3a with cytoplasmic stress granules.

Table 1
Effect of oxidative stress on the nuclear and cytoplasmic levels of ERK1/2, Akt and FoxO3a. Results for the quantification of fluorescence intensities in nuclear (Nuc) and cytoplasmic (Cyt) compartments of stressed cells are summarized. The distribution of proteins in DEM-treated cells is compared to unstressed controls. The kinase concentrations in the nuclear and cytoplasmic compartments of untreated cells are defined as 1; all changes are expressed relative to the levels in unstressed controls. +++, increase to 2.1–2.9-fold; ++, increase to 1.5–2.0-fold; +, increase to 1.2–1.4-fold; +/−, 0.8–1.1-fold change in kinase levels. Note that the profile of changes in nuclei is similar for p-ERK1/2, pAkt-473 and FoxO3a.

Kinase	DMSO		LY294002		PD98059		LY294002 + PD98059	
	Nuc	Cyt	Nuc	Cyt	Nuc	Cyt	Nuc	Cyt
p-ERK1/2	++	+/−	+++	+	+	+/−	++	+/−
t-ERK1/2	+	+/−	++	+/−	+	+/−	+	+/−
p-Akt308	++	++	NA	NA	++	+++	NA	NA
p-Akt473	+++	++	+++	++	++	++	+++	++
t-Akt	+	++	+/−	+	+/−	+	+	++
FoxO3a	++	+	+++	++	++	+	+++	++

4. Discussion

With the experiments described here we advanced the understanding of the complex regulatory mechanisms that underlie the localized action of signaling events. To our knowledge, this is the first study that applies newly developed methods in confocal microscopy combined with computer-based image-analysis to provide a quantitative investigation of the activation and localiza-

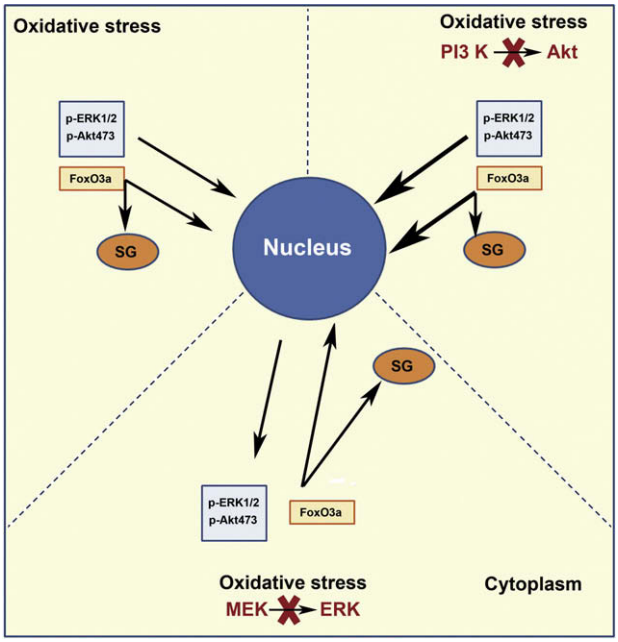

Fig. 4. Simplified model for stress-induced changes in the distribution of p-ERK1/2, p-Akt473 and FoxO3a. Arrows indicate changes in the nuclear levels of p-ERK1/2, p-Akt473 and FoxO3a. Protein kinase inhibition reveals that in stressed cells cross-talk between PI3 kinase and MEK pathways controls the nucleocytoplasmic distribution of p-ERK1/2 and p-Akt473. Moreover, FoxO3a association with different cellular compartments depends on oxidant exposure and kinase activation. Overall, the model proposes that compartmental changes in p-ERK1/2 and p-Akt473 kinase levels, together with cross-talk between both signaling modules, may modulate the nucleocytoplasmic distribution of numerous target molecules, including FoxO3a.

tion of Akt and ERK1/2 kinases under normal and oxidative stress conditions. Our research tested the hypothesis that oxidative stress alters the subcellular distribution of these kinases, possibly generating specific pools of signal transducers that may determine the end-point result of signaling events. Furthermore, we demonstrate that PI3 kinase activation affects MEK-dependent processes and vice versa, in line with the idea that interplay between both pathways participates in the control of signaling in response to oxidant exposure. At the molecular level, several mechanisms will contribute to the steady-state distribution of Akt, ERK1/2 and their target FoxO3a. This includes the modification by upstream kinases or phosphatases, trafficking in and out of the nucleus as well as retention in the nuclear or cytoplasmic compartment. The simplified model shown in Fig. 4 depicts the stress-induced changes for p-ERK1/2, p-Akt473 and the transcription factor FoxO3a, showing that these changes were distinct in the nuclear and cytoplasmic compartments. In particular, in stressed cells a striking correlation was detected between *nuclear* p-ERK1/2 and p-Akt473 levels, indicating that signaling in the nucleus through both pathways is linked. These results suggest that in oxidant-treated cells PI3 kinase and MEK → ERK1/2 signaling are interdependent events. Thus, inhibition of PI3 kinase signaling not only promotes ERK1/2 activation; it also leads to phosphorylation of Akt on Ser473.

It should be noted that in stressed cells treatment with LY294002 was able to override completely the effect of PD98059 for nuclear p-Akt473, but only partially for nuclear p-ERK1/2. The reasons for this difference are not understood, but as a consequence the nuc/cyt ratio was lower for p-ERK1/2 than for p-Akt473. Different not mutually exclusive scenarios may explain these results. For instance, MEK activity in cells treated with both inhibitors may be sufficient to generate p-Akt473 to an extent similar to LY294002-treated cells. Alternatively, other factors that determine the amount of p-Akt473 and p-ERK1/2 in nuclei may respond differently to the simultaneous addition of LY294002 and PD98059. Possible candidates are one or more of the phosphatases that regulate the levels of p-Akt473 or p-ERK1/2 [30–32].

Interestingly, in our studies PI3 kinase inhibition had distinct effects on Thr308 and Ser473 modification of Akt, which may be explained by the complex regulation of Akt phosphorylation. Whereas PDK1, a downstream target of PI3 kinase, phosphorylates Thr308, different pathways are implicated in the modification of Ser473, some of which are insensitive to inhibitors of PI3 kinase [8–15]. Based on the results described here we propose that PI3 kinase independent signaling plays a major role in modifying this Ser473 in our experimental settings, leading to an increase in Ser473 phosphorylation when Thr308 modification is abolished.

The cross-talk identified by us is likely to have intricate consequences for signal transduction. For instance, Ser473 phosphorylation of Akt is believed to stabilize the active conformation of the kinase [8], and p-Akt308 may differ in substrate specificity from Akt dually modified on Thr308 and Ser473 [8,9]. As such, Akt phosphorylated on both Thr308 and Ser473 seems to favor pro-survival signaling, in part by promoting FoxO phosphorylation in the nucleus [9]. In addition to the phosphorylation of Akt on Thr308 and Ser473, the appropriate nucleocytoplasmic distribution of the kinase will be crucial for downstream events. The proper localization will affect the phosphorylation not only of nuclear targets, but of cytoplasmic substrates as well. These cytoplasmic targets include, but are not limited to, components that modulate the organization and functions of the cytoplasmic compartment, such as actin and proteins involved in translational control.

The principles described for PI3 kinase inhibition also apply to signaling through MAPKs, and it is noteworthy that MEK inhibition altered the levels of p-Akt308 and p-Akt473 in stressed cells. Specifically, PD98059 increased the concentration of p-Akt308 in both nuclei and cytoplasm, whereas the amount of p-Akt473 in nuclei was reduced under stress and normal conditions. These observations indicate that MEK activities impinge differently on the phosphorylation of Akt residues 308 and 473, adding further complexity to the interplay between PI3 and MEK1/2 and MEK5 signaling pathways, consistent with reports that suggested cross-talk between Raf and Akt signaling [22]. However, data presented here go beyond previous studies as they provide a quantitative analysis of this interplay between Akt and ERK1/2 signaling events by measuring the compartment-specific changes in kinase distribution. This enabled us to identify a new link in the nucleus between p-ERK1/2 and p-Akt473. Recent studies by others demonstrate the importance of Akt signaling in the cytoplasm to achieve a specific physiological response [16]. Our data now provide evidence for specific signaling events and interplay between signaling cascades that are located in the nuclear compartment and are likely to impact numerous nuclear processes.

In conclusion, on the basis of the results described here, we propose that upon oxidative stress, signaling through PI3 kinase and MEK occurs in an interdependent fashion. The balance between both pathways will be critical to define the endpoint of the cellular response and ultimately the survival under oxidative stress conditions.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2009.05.011.

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